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Studies on the genus *Paecilomyces* in China. Application of DELTA expert system on the entomopathogenic *Paecilomyces* sensu lato

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Abstract — The DELTA expert system was used for the first time to study the genus *Paecilomyces*. A DELTA database of twenty-four entomopathogenic *Paecilomyces* in China was established and successfully used to create a key, natural language taxon descriptions, a phenetic tree, and an interactive identification system. These results will provide an effective platform for the exchange of information with both domestic and foreign workers.

Key words — entomopathogenic fungi, numerical classification

Introduction

The classical methods of taxon identification and subsequent preparation of keys and characteristic descriptions involve time-consuming and laborious procedures. At the same time, the order and content of descriptions are often inconsistent, with some items even omitted, which can make identifications unreliable. To meet this problem, several expert software systems are emerging. One of the most representative is the DELTA (DEscription Language for TAxonomy) expert system software (Chen & Kuoh 2000a, b; Chen 2003, 2004). It has been selected as the standard for the biological taxonomy data by the international taxonomic database working group (Dallwitz 2000a, b).

The purpose of this study is to establish the DELTA database of entomopathogenic *Paecilomyces* sensu lato in China and explore its functional extensions.

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Materials and methods

Materials

A *Paecilomyces* DELTA database was constructed for the species listed in Table 1 using information mainly from the Institute of Fungal Resources, Guizhou University, along with other world reports.

Table 1. Entomopathogenic *Paecilomyces* spp. used for constructing the DELTA database.

Name	Reference
Paecilomyces amoeneroseus (Henn.) Samson	Samson 1974, Liu & Liang 2003
P. atrovirens Z.Q. Liang & A.Y. Liu	Liang et al. 2003
P. breviramosus Bissett	Tzean et al. 1997
P. cateniannulatus Z.Q. Liang	Liang 1981
P. cateniobliquus Z.Q. Liang	Liang 1981
P. cicadae (Miq.) Samson	Samson 1974, Liang et al. 2003, Chen 1991
P. farinosus (Holmsk.) A.H.S. Br. & G. Sm.	Samson 1974, Tzean et al. 1997, Liang 1981, Brown & Smith 1957, Li et al., 2003
P. fumosoroseus (Wize) A.H.S. Br. & G. Sm.	Samson 1974, Tzean et al. 1997, Brown & Smith 1957, Li et al. 2003
P. fumosoroseus var. *beijingensis* Q.X. Fang & Q.T. Chen	Fang et al. 1983
P. griseoviridis M.X. Dai	Dai 1998
P. gunnii Z.Q. Liang	Liang 1985
P. gunnii var. *minor* Z.Z. Li et al.	Li et al. 1999
P. javanicus (Friedr. & Bally) A.H.S. Br. & G. Sm.	Samson 1974, Brown & Smith 1957
P. lilacinus (Thom) Samson	Samson 1974, Li et al. 2003.
P. loushanensis Z.Q. Liang & A.Y. Liu	Liang et al. 1997
P. marquandii (Massee) S. Hughes	Samson 1974, Brown & Smith 1957
P. militaris Z.Q. Liang	Liang 2001
P. nostocoides M.T. Dunn	Michael 1983
P. odonatae Zuo Y. Liu et al.	Liu et al. 1995–96
P. rariramus Z.Q. Liang & B. Wang	Liang et al. 2002
P. sinensis Q.T. Chen et al.	Chen et al. 1984
P. suffultus (Petch) Samson	Samson 1974
P. tenuipes (Peck) Samson	Samson 1974, Tzean et al. 1997
P. xylariiformis (Lloyd) Samson	Samson 1974

Methods

The DELTA system was presented with data from the selected *Paecilomyces* strains, and its functions were used to prepare a key, natural language taxon descriptions, a phenetic tree, and an interactive identification system (Chen et al. 2003; Aiken et al. 1996; Dallwitz et al. 1995a, b; Dallwitz 1974, 1980; Heywood 1979).

Results

Establishment of DELTA database for tested *Paecilomyces* sensu lato

Twenty important characters of *Paecilomyces* were coded acccording to the requirements of the DELTA system. The characters and character states are listed below in Table 2.

Table 2. Characters and character states in *Paecilomyces*.

#1. Synnemata/
1. present/
2. absent/

#2. Colony < colour1>/
1. light/
2. coloured/

#3. Colony <color 2>/
1. green/
2. purple/
3. pink/
4. red/

#4. Reverse <colour1>/
1. light/
2. brown/
3. red/
4. green/

#5. Conidiophore <smooth or rough>/
1. smooth/
2. rough/

#6. <Conidiophore1>/
1. complex/
2. not complex/

#7. <conidiophore 2>/
1. absent/
2. simple/

#8. Phialides at the basal portion <Shape1>/
1. cylindrical/
2. ellipsoidal/
3. subglobose/
4. lecythiform/

#9. <Mode1>/
1. solitary/
2. not solitary

#10. Phialides Length/um long/

#11. Phialides Width/um wide/

#12. Conidia <Surface>/
1. smooth/
2. rough/

#13. Conidia <type>/
1. with one type/
2. with two different types /

#14. Conidia <Shape1>/
1. subglobose/
2. ellipsoidal/
3. fusiform/
4. reniform/
5. triangular/
6. cylindrical/
7. clavate/

#15. Conidia Length/um long/

#16. Conidia Width/um wide/

#17. Conidial chain <Arrangement>/
1. straight /
2. imbrate/
3. straight or in head/

#18. Chlamydospore <Present or Absent>/
1. present/
2. absent/

#19. Host<1>/
1. general insect /
2. special animal /

#20. Host<2>/
1. Nematode/
2. Odonata

Key to Chinese entomopathogenic *Paecilomyces*

1	Colony light	2
	Colony dark	16
2	Phialides at the basal portion cylindrical	3
	Phialides at the basal portion not cylindrical	8
3	Conidial chain straight	4
	Conidial chain not straight	6
4	Reverse light; Synnemata present; Conidia smooth; not ellipsoidal	*P. javanicus*
	Reverse dark; Synnemata absent; Conidia rough; ellipsoidal	5
5	Chlamydospore present	*P. gunnii*
	Chlamydospore absent	*P. gunnii* var. *minor*
6	Conidia subglobose; straight or in head	*P. militaris*
	Conidia not subglobose; imbricate	7
7	Conidia ellipsoidal	*P. cateniobliquus*
	Conidia not ellipsoidal	*P. loushanensis*
8	Conidia ellipsoidal	9
	Conidia not ellipsoidal	14
9	Conidial chain straight	10
	Conidial chain not straight	13
10	Conidiophore complex	11
	Conidiophore not complex	12
11	Reverse light	*P. farinosus*
	Reverse dark	*P. sinensis*
12	Conidia ellipsoidal	*P. xylariiformis*
	Conidia not ellipsoidal	*P. suffultus*
13	Conidia chain imbricate; Conidia with one type; Host: not *Odonata*	*P. cateniannulatus*
	Conidia chain straight or in head; Conidia with two different types; Host: *Odonata*	*P. odonatae*
14	Conidia subglobose; not complex	*P. rariramus*
	Conidia not subglobose; complex	15
15	Conidia ellipsoidal	*P. breviramosus*
	Conidia not ellipsoidal	*P. tenuipes*
	Conidia not ellipsoidal	*P. cicadae*
16	Reverse light	17
	Reverse dark	20
17	Colony purple; Nematode	18
	Colony not purple; general insect	*P. fumosoroseus*
	Colony not purple; general insect	*P. fumosoroseus* var. *beijingensis*

18 Conidia with one type; Conidia not subglobose 19
Conidia with two different type; Conidia subglobose ..... *P. nostocoides*

19 Conidiophore smooth; Chlamydospore present ..... *P. marquandii*
Conidiophore rough; Chlamydospore absent ..... *P. lilacinus*

20 Cylindric at the basal portion of Phialides ..... *P. griseoviridis*
Not cylindrical at the basal portion of Phialides 21

21 Synnemata present; complex; Conidia subglobose; conidial chain straight ..... *P. amoeneroseus*
Synnemata absent; not complex; Conidia not subglobose; conidial chain not straight ..... *P. atrovirens*

This key was fast and simple to use. From the key contents, these entomopathogenic *Paecilomyces* were primarily differentiated by colony color, shape of phialides at the base, shape and aggregation form of conidia, and host specificity.

Natural language descriptions

Some examples are as follows:

P. cateniobliquus Z.Q. Liang

Synnemata present. Colony 30 mm, pink, floccose. Reverse orange. Mycelium smooth, 1–1.5 μm. Conidiophore smooth, simple, 90–150 μm long, 1–1.5 μm wide. Phialides at the basal portion cylindrical or subglobose to globose; or on the branched conidiophore, in a whorl of 2–4 phialides. Phialides 8.5–12 μm long, 1–1.5 μm wide. Neck 0.5 μm wide. Conidia smooth, hyaline, with one type, ellipsoidal or cylindrical, 2.5–12 μm long, 1–2.5 μm wide. Conidial chain imbricate. Chlamydospore absent. Mesophilic.

Teleomorph: *Cordyceps*.

Habit: *Lepidoptera*.

P. militaris Z.Q. Liang

Synnemata present. Colony 50 mm, yellowish to ecru-olive or orange, floccose. Reverse yellow. Mycelium smooth. Conidiophore smooth, simple. Phialides at the basal portion cylindrical; or on the branched conidiophore, in a whorl of 3–5 phialides. Phialides 6–20 μm long, 0.5–1.5 μm wide. Neck 0.5 μm wide. Conidia smooth, hyaline, with one type, subglobose or globose, 1–3 μm long, 1.5–3 μm wide. Conidial chain straight or in head. Chlamydospore absent. Mesophilic.

Teleomorph: *Cordyceps*.

Habit: Various insects of *Lepidoptera*.

P. odonatae Zuo Y. Liu, Z.Q. Liang & A.Y. Liu

Colony 55–63 mm. Colony white, floccose. Reverse yellow. Mycelium smooth. Conidiophore smooth, complex. Phialides at the basal portion ellipsoidal or awl-shaped,

solitary; or on the branched conidiophore, in a whorl of 2–4 phialides. Phialides 4.8–17 μm long, 1.8–2.4 μm wide. Conidia smooth, hyaline, with two different types, ellipsoidal or cylindrical or fusiform, 2.4–4.2 μm long, 1.7–2.2 μm wide. Conidial chain straight or in head. Chlamydospore absent. Mesophilic.

Teleomorph: *Cordyceps*.

Habit: *Odonatae*.

Although some of the DELTA descriptions needed small editorial modifications, this method resulted in a great saving of time and energy for text input.

Numerical taxonomy analysis

Distance matrix generated by DELTA system: Distance coefficients and distance locations between 24 operation taxonomic units (OUT) were calculated and sorted using all 20 characters. The majority of the distance coefficients were greater than 0.03, and the DELTA system distance matrix distinguished their differences. It also provided the basic data for generation of a phenetic tree and other systematic analysis.

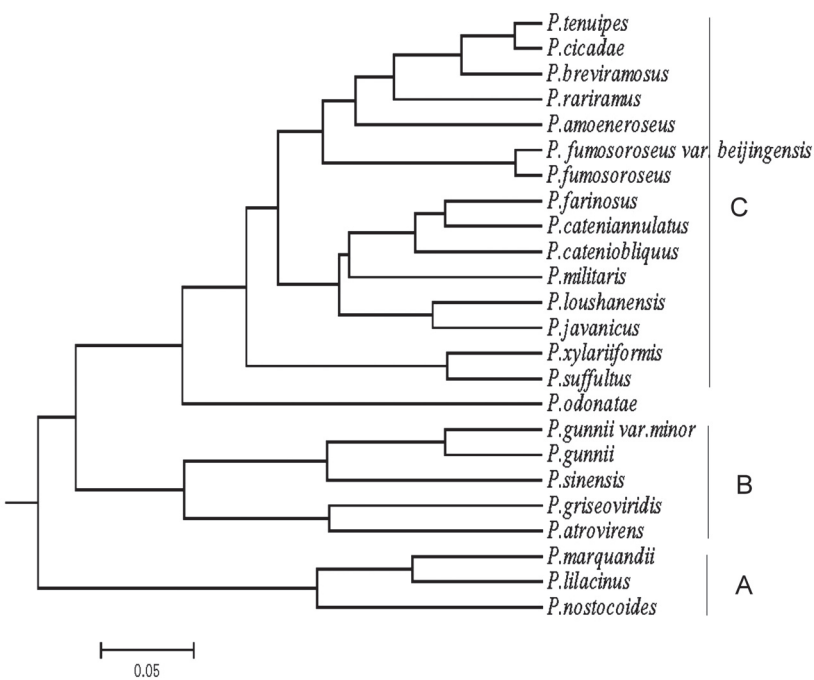

Fig. 1 Phenetic tree from Chinese twenty-four entomopathogenic *Paecilomyces* species by the DELTA system

Phenetic tree generated by DELTA system: Numerical classification does not normally place priority weighting on characters. In this study all twenty chosen characters were given equal value and not weighted. The phenetic tree based on morphological characters (Fig. 1) shows species clearly divided into three groups.

Characters shared among the three group A species are purple colony color, oval-shape phialides base, straight-chain conidia, and parasitizing nematodes, indicating that host specificity (e.g., insects, nematodes) is an important character in entomopathogenic *Paecilomyces* classification.

Shared characters among the five group B taxa are a greenish colony color with a brown reverse, straight-chain conidia, and parasitizing insects. In early classification, *Paecilomyces* never explicitly appeared really green. The phenetic tree established by DELTA system supports to a certain extent that colors are significant in differentiating *Paecilomyces* species.

The fifteen group C taxa share bright colors, produce synnemata, and parasitize insects. The tree shows *P. odonatae* as a separate species very close to group C; it is characterized by two (cylindrical and oval) spore types and a dragonfly host (unique within *Paecilomyces*). The phenetic tree truly reflected the unique character.

Interactive identification system

The system provided a useful experts system platform for professional workers and other related researchers.

Discussion

Bainier established the form genus *Paecilomyces* in 1907 based on morphological and biological characters with *P. variotii* Bainier as type species. Samson (1974) recognized and described in detail 31 species which he divided into sect. *Paecilomyces* and sect. *Isarioidea*. With the development of molecular techniques, the phylogenetic analysis of *Paecilomyces* had made a great progress (Han et al. 2005, 2007; Liang et al. 2007). Luangsa-ard et al. (2004), who studied the phylogenetic relationships in *Paecilomyces* sensu lato, showed that *Paecilomyces* was polyphyletic across two subclasses, *Sordariomycetidae* and *Eurotiomycetidae*. Analysis of the phylogenetic relationships of *Paecilomyces* sect. *Isarioidea* species revealed that entomopathogenic species with *Cordyceps* teleomorphs were monophyletic and designated as members of the genus *Isaria* (Luangsa-ard et al. 2005). However, some entomopathogenic species — such as *P. lilacinus, P. marquandii, P. gunnii*, and *P. odonatae* (Luangsa-ard et al. 2005; Liang 1985; Liu et al. 1995–96) — not yet included in the genus *Isaria* need further study to determine their true relationships with one another. So *Paecilomyces* spp. in the broad sense were used in our study.

Handling documents, samples, data, and other information is very time-consuming and laborious regardless of the particular perspectives and methods adapted by individual taxonomists. After Liu (2006) suggested that computer databases and expert systems could effectively reduce conventional information processing and improve efficiency, adoption of computerized analyses were eagerly anticipated by taxonomists,

The DELTA expert system is a rapid procedural operation, and has many other advantages, such as diverse functions that enable the morphological characters to be routinely standardized digitally and thus suitable for international communication (Li 1996). These advantages allow building of databases to ensure complete and satisfactory results. The selection of the characters and states is closely related, so that only by establishing a complete and accurate database is it possible to produce high-quality function extensions. To achieve this, researchers must carefully find, analyze, and select characters. Constructing of a database requires accurate character states and uniform terminology. Any inconsistent and nonstandard terms used in descriptions will hinder the accuracy of identification. Traditional classification often employs qualitative adverbs such as "longer…, wide…, narrow…" and so on that depend on visual interpretations by different researchers and so are unlikely to be identical. Vague descriptions will hence affect identification. Uniform terms require researchers to be familiar with the classification group, and should be summarized to include as much information as possible.

A database of the Chinese entomopathogenic *Paecilomyces* was created successfully in this study. It also produced a key, natural language descriptions, an interactive identification system, and a phenetic tree. However, characters useful for diagnosis in classical identification, such as the form of conidial aggregation, are not revealed by the phenetic tree because the states are unweighted and given equal treatment by the DELTA system.

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